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Verification of Commercial Decontamination Technologies in Bench-Scale Studies Using *Bacillus anthracis* Spores

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Outline

- Purpose of Testing
- Technologies Tested
- Test Apparatus
- Test Materials & Organisms
- Parameters Evaluated
- Generalized Test Procedures
- BIOQUELL, Inc. – Hydrogen Peroxide Gas Testing
- CERTEK, Inc. – Formaldehyde Gas Testing
- CDG Technology, Inc. – Chlorine Dioxide Testing
- Acknowledgements

Purpose of Testing

- EPA ETV Program – Battelle, Testing Contractor
  - Verify the performance characteristics of environmental technologies and report objective information to permitters, buyers and prospective users
  - Testing performed as stipulated in test/quality assurance plans developed with the participation of technical experts, stakeholders and vendors
- Focus of Initial Tests
  - Verify performance of fumigant-type technologies for decontaminating indoor surfaces inoculated with *B. anthracis* (Ames) and surrogates

Configuration of Testing Apparatus

Technology being tested

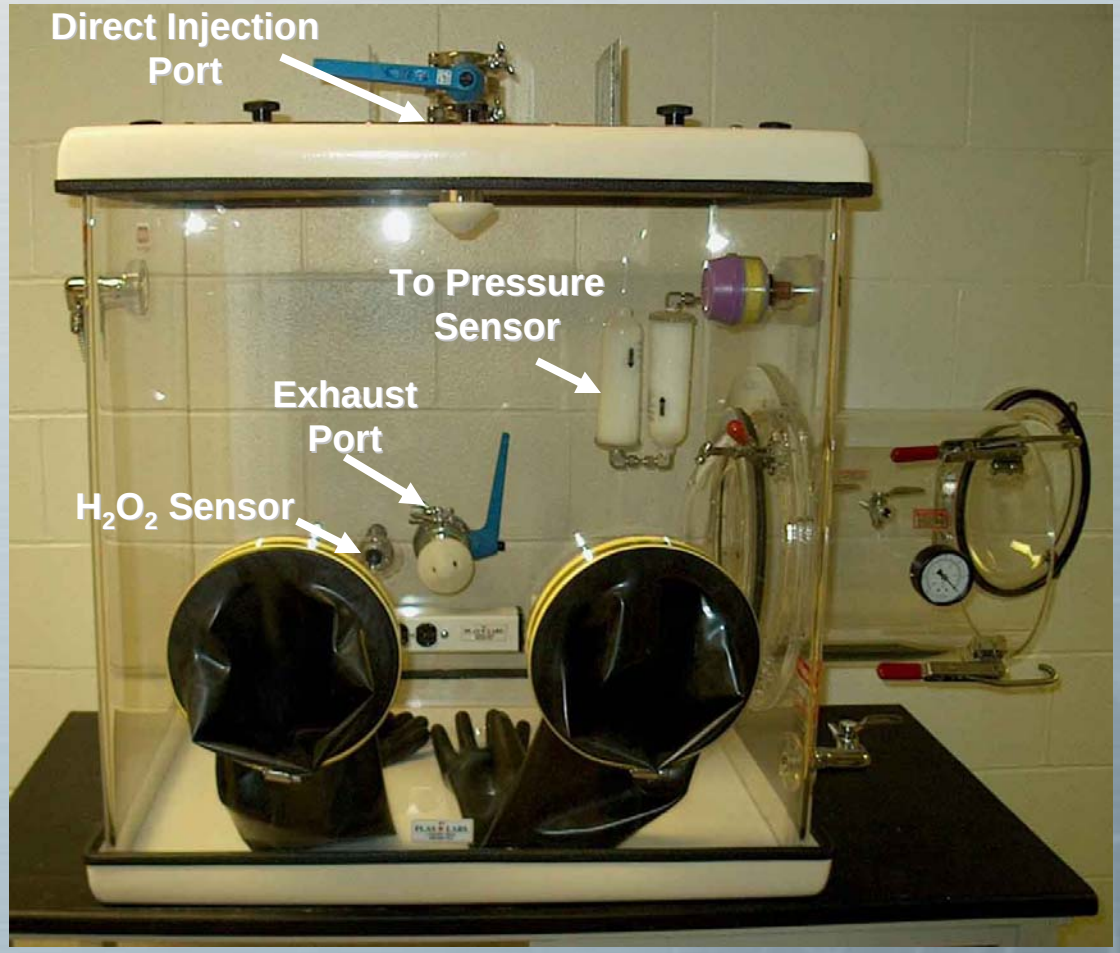
Plas-Labs Glove Box

BL-3 Laboratory

Test Chamber

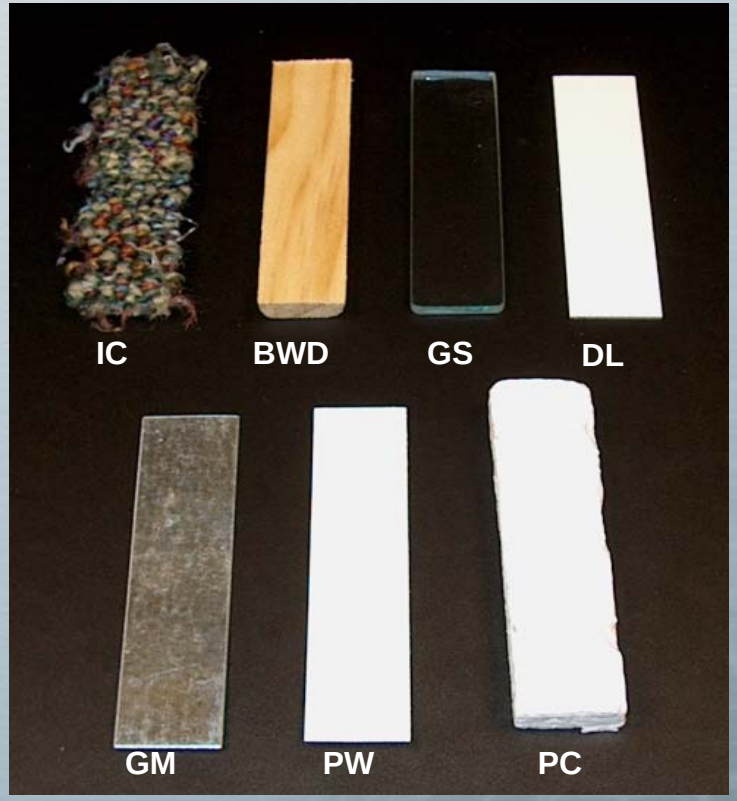
Plas-Labs Compact Glove Box

modified per vendor's request (BIOQUELL configuration shown)



Test Materials

- Industrial-grade carpet (IC)
- Bare wood (pine lumber) (BWD)
- Glass (GS)
- Decorative laminate (DL)
- Galvanized metal ductwork (GM)
- Painted (latex, flat) wallboard paper (PW)
- Painted (latex, semi-gloss) concrete cinder block (PC).

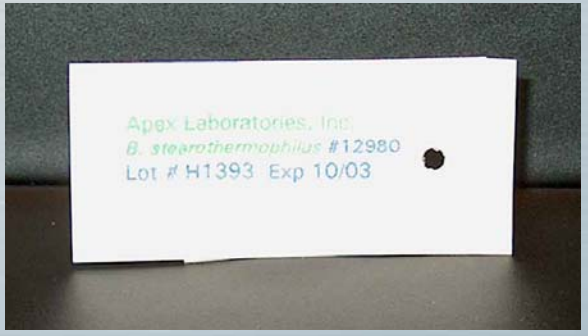


Organisms

- *Bacillus anthracis* Ames
- *Bacillus subtilis* (ATCC 19659)
- *Geobacillus stearothermophilus* (ATCC 12980)

- Biological Indicators
  - *Bacillus subtilis* (ATCC 19659)
  - *Geobacillus stearothermophilus* (ATCC 12980)

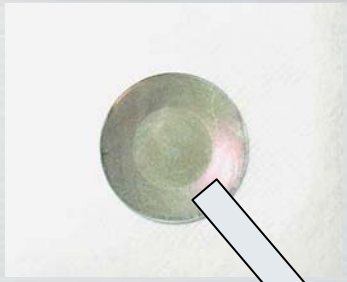
- Spore Strips
  - *Bacillus atrophaeus* (ATCC 9372)



Biological Indicator

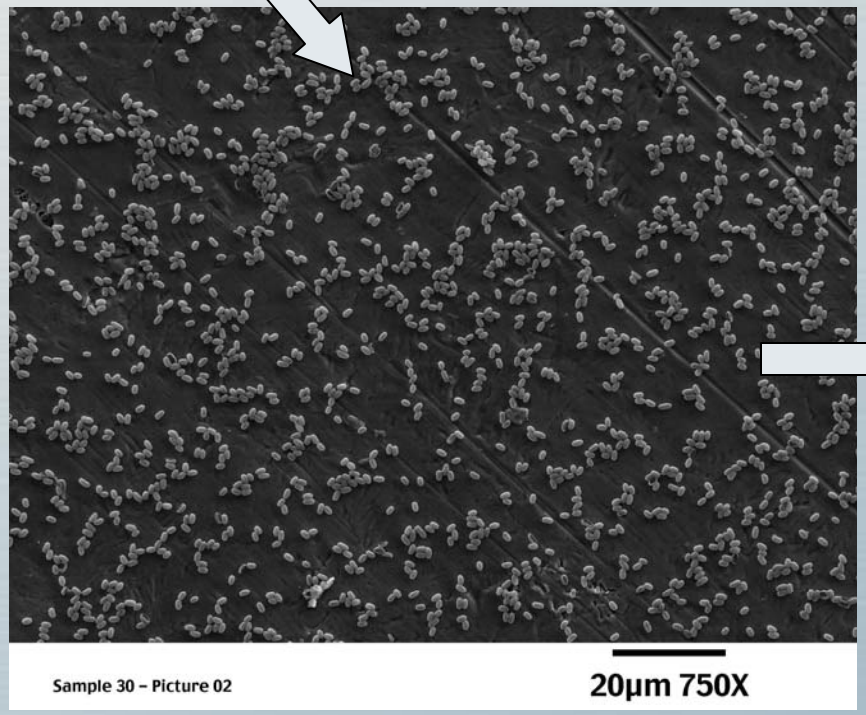
Spore Strip

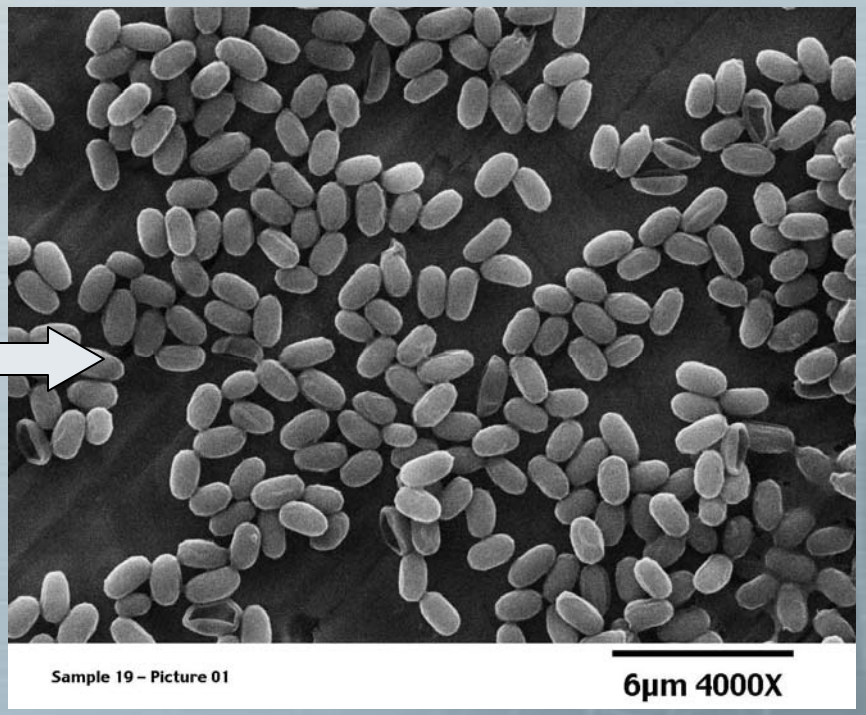
Organisms



Biological Indicators:

SEM Images Courtesy of
Apex Laboratories, Inc.





Parameters Evaluated

- Biological Efficacy Test
  - Log Reduction in viable spores on test materials
    - Quantitative
  - Positive/Negative bacterial growth at 1 and 7 days
    - Biological indicators/Spore strips; qualitative
- Coupon Damage
  - Changes in appearance, color, texture, etc.

Generalized Test Procedure

- Couple decontamination technology to test chamber
- Prepare coupons of test materials, inoculate
- Place into test chamber
- Implement decontamination technology
- Remove coupons from test chamber
- Analyze

Analysis Procedure for *B. anthracis* Ames

- Procedure

Extract – 15 min

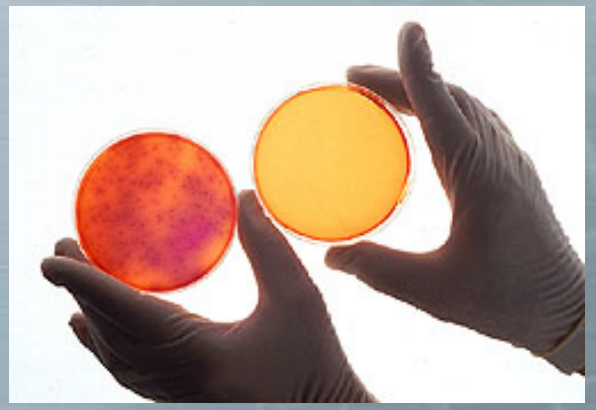
***0.1% Triton X-100 in PBS**

Orbital shaker at 200 rpm

Supernatant

Heat Shock for 1 hr at 65ºC

Dilution plating



Enumeration

Test Material

+

TSB

Growth Assessment at 1 and 7 days

Data Analysis

- Efficacy (E) Calculation

  E = log(N'/N)

  N' = total viable spores recovered from control samples (no decon)

  N = total viable spores recovered from decontaminated samples

- Data (total spores; percent recovery)
- Expressed as Mean ± SD

BIOQUELL, Inc. – Hydrogen Peroxide

Cycle Parameters
(Provided by Vendor)

- Cycle pressure: 20 Pascals
- Conditioning time: 10 min
- Gassing time: 20 min
- Gassing dwell: 20 min
- H_2O_2 injection rate: 2.0 g/min
- H_2O_2 dwell rate: 0.5 g/min
- H_2O_2 concentration during dwell: $\geq$1000 ppm
- Aeration time: set for 9999 min

CLARUS™ C Unit

BIOQUELL, Inc. – Hydrogen Peroxide

Microcondensation during Dwell Phase

BIOQUELL, Inc. – Hydrogen Peroxide Results

Mean Efficacy for Spores

Material[a]		*B. anthracis*[b]	*B. subtilis*[b]	*G. stearothermophilus*[b]
Porous	**Industrial-grade Carpet**	3.01 (2.62-3.55)[c]	1.63 (1.46-1.76)[c, d]	0.81 (0.69-0.89)[d]
	Painted Concrete	6.36 (3.92-7.58)[c]	6.09 (5.58-7.10)[c]	4.09 (3.09-5.15)[c, d]
	Bare Wood	3.70 (3.20-4.46)[c]	2.18 (1.81-2.75)[c, d]	4.09 (3.80-4.61)[c]
Non-porous	**Glass**	≥7.92 (7.92)[c]	≥7.57 (7.57)[c]	4.68 (4.27-5.11)[c, d]
	Decorative Laminate	≥7.85 (7.85)[c]	≥7.66 (7.66)[c]	3.75 (2.20-4.77)[c, d]
	Painted Wallboard Paper	≥6.92 (6.92)[c]	≥7.52 (7.52)[c]	5.98 (5.47-6.99)[c]
	Galvanized Metal Ductwork	≥7.54 (7.54)[c]	6.44 (5.73-7.56)[c]	1.97 (1.90-2.04)[c, d]

[a] Three replicates were used for each test material for each organism.
[b] Log reduction in spores with range in parentheses.
[c] Mean significantly different from 0 (P≤0.05).
[d] Surrogates significantly different from *B. anthracis* for specified material (P≤0.05).

BIOQUELL, Inc. – Hydrogen Peroxide Statistical Analysis

Material		*B. anthracis*	*B. subtilis*	*G. stearothermophilus*
Porous	**Industrial-grade Carpet**	3.01	1.63	0.81
	Painted Concrete	6.36	6.09	4.09
	Bare Wood	3.70	2.18	4.09
Non-porous	**Glass**	7.92	7.57	4.68
	Decorative Laminate	7.85	7.66	3.75
	Painted Wallboard Paper	6.92	7.52	5.98
	Galvanized Metal Ductwork	7.54	6.44	1.97

All values are significantly different than zero ($P \leq 0.05$) except [ ]

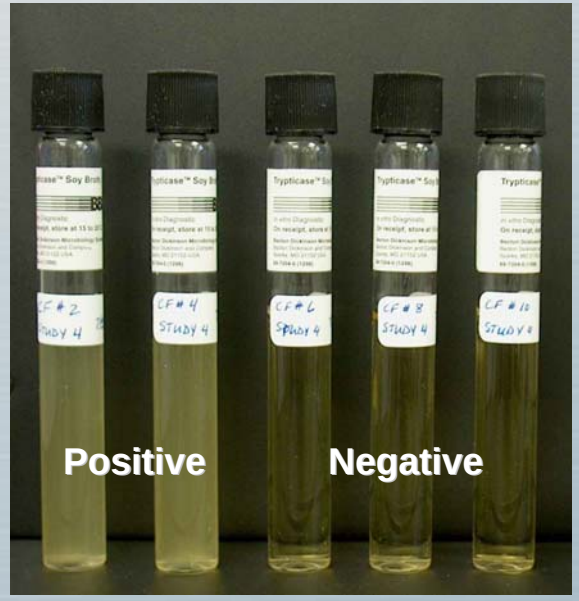
BIOQUELL, Inc. – Hydrogen Peroxide Statistical Analysis

Material		*B. anthracis*	*B. subtilis*	*G. stearothermophilus*
Porous	**Industrial-grade Carpet**	3.01	1.63	0.81
	Painted Concrete	6.36	6.09	4.09
	Bare Wood	3.70	2.18	4.09
Non-porous	**Glass**	7.92	7.57	4.68
	Decorative Laminate	7.85	7.66	3.75
	Painted Wallboard Paper	6.92	7.52	5.98
	Galvanized Metal Ductwork	7.54	6.44	1.97

Mean value is significantly different than *B. anthracis* (P=0.05)

BIOQUELL, Inc. – Hydrogen Peroxide Growth Indicators

Indicator (Organism)		Day 1			Day 7		
		S1	S2	S3	S1	S2	S3
Biological Indicator (*B. subtilis* ATCC 19659)	Control	+	+	+	+	+	+
Biological Indicator (*G. stearothermophilus* ATCC 12980)	Control	+	+	+	+	+	+
Spore Strip (*B. atrophaeus* ATCC 9372)	Control	+	+	+	+	+	+
Biological Indicator (*B. subtilis* ATCC 19659)	Decontaminated	-	-	-	-	-	-
Biological Indicator (*G. stearothermophilus* ATCC 12980)	Decontaminated	-	-	-	-	-	-
Spore Strip (*B. atrophaeus* ATCC 9372)	Decontaminated	-	-	-	-	-	-



For all tests, control biological indicators and spore strips displayed positive growth while those decontaminated showed negative growth.

CERTEK, Inc. – Formaldehyde

Cycle Parameters
(Provided by Vendor)

- Cycle pressure: ambient
- Conditioning time: 1 hour
  (ramp-up of formaldehyde concentration)
- Dwell time: 10 hours
- Formaldehyde concentration: theoretical – 8600 ppm
  actual – 1,100 ppm
- Relative humidity: 75%
- Neutralization: 1 hour
  (ammonium carbonate)
- Total run time: 16-18 hours

CERTEK, Inc. Unit

CERTEK, Inc. – Formaldehyde Results

Mean Efficacy for Spores

Material[a]		*B. anthracis*[b]	*B. subtilis*[b]	*G. stearothermophilus*[b]
Porous	**Industrial-grade Carpet**	≥7.00 (7.00)[c]	≥8.04 (8.04)[c]	5.68 (4.81-7.18)[c, d]
	Painted Concrete	7.15 (5.93-7.76)[c]	6.02 (5.61-6.22)[c]	6.20 (4.03-7.29)[c]
	Bare Wood	≥7.61 (7.61)[c]	6.58 (5.57-7.08)[c]	≥6.82 (6.82)[c]
Non-porous	**Glass**	≥7.71 (7.71)[c]	≥7.79 (7.79)[c]	≥7.24 (7.24)[c]
	Decorative Laminate	6.47 (5.61-7.66)[c]	7.29 (6.38-7.74)[c]	≥7.12 (7.12)[c]
	Painted Wallboard Paper	≥5.17 (5.17)[c]	≥7.68 (7.68)[c, d]	≥7.19 (7.19)[c, d]
	Galvanized Metal Ductwork	≥7.86 (7.86)[c]	6.24 (5.39-7.87)[c, d]	≥7.64 (7.64)[c]

[a] Three replicates were used for each test material for each organism.
[b] Log reduction in spores with range in parentheses.
[c] Mean significantly different from 0 (P≤0.05).
[d] Surrogates significantly different from *B. anthracis* for specified material (P≤0.05).

CERTEK, Inc. – Formaldehyde Statistical Analysis

Material		*B. anthracis*	*B. subtilis*	*G. stearothermophilus*
Porous	**Industrial-grade Carpet**	≥7.00	≥8.04	5.68
	Painted Concrete	7.15	6.02	6.20
	Bare Wood	≥7.61	6.58	≥6.82
Non-porous	**Glass**	≥7.71	≥7.79	≥7.24
	Decorative Laminate	6.47	7.29	≥7.12
	Painted Wallboard Paper	≥5.17	≥7.68	≥7.19
	Galvanized Metal Ductwork	≥7.86	6.24	≥7.64

All values are significantly different than zero ($P \leq 0.05$) except [ ]

CERTEK, Inc. – Formaldehyde Statistical Analysis

Material		*B. anthracis*	*B. subtilis*	*G. stearothermophilus*
Porous	**Industrial-grade Carpet**	≥7.00	≥8.04	5.68
	Painted Concrete	7.15	6.02	6.20
	Bare Wood	≥7.61	6.58	≥6.82
Non-porous	**Glass**	≥7.71	≥7.79	≥7.24
	Decorative Laminate	6.47	7.29	≥7.12
	Painted Wallboard Paper	≥5.17	≥7.68	≥7.19
	Galvanized Metal Ductwork	≥7.86	6.24	≥7.64

Mean value is significantly different than *B. anthracis* (P=0.05)

CDG Research Corporation – Chlorine Dioxide

Cycle Parameters
(Provided by Vendor)

- Cycle pressure: ambient
- Conditioning time: N/A
- Dwell time: 6 hours
- Chlorine Dioxide concentration: 2000 ppm
- Relative humidity: 70-80%
- Temperature: 23-27ºC
- Neutralization: 30-60 min followed by overnight aeration (10% NaOH, 10% NaS_2O_4 in water)
- Total run time: 16-18 hours

CDG Unit

CDG Research Corporation – Chlorine Dioxide Results

Mean Efficacy for Spores

Material[a]		*B. anthracis*[b]	*B. subtilis*[b]	*G. stearothermophilus*[b]
Porous	**Industrial-grade Carpet**	4.62 (4.11-5.50)	4.44 (4.28-4.62)	3.22 (3.17-3.28)[c]
	Painted Concrete	7.25 (6.24-7.76)	4.74 (4.44-4.93)[c]	5.79 (5.08-6.90)[c]
	Bare Wood	4.33 (4.10-4.48)	4.48 (4.14-4.79)	3.79 (3.70-3.87)
Non-porous	**Glass**	5.70 (5.35-6.06)	5.23 (4.89-5.49)	3.87 (3.64-4.20)[c]
	Decorative Laminate	4.57 (4.19-4.85)	5.14 (4.83-5.34)	4.44 (4.29-4.59)
	Painted Wallboard Paper	≥7.68 (7.68)	4.62 (3.24-5.47)[c]	5.62 (4.65-6.87)[c]
	Galvanized Metal Ductwork	≥7.79 (7.79)	5.57 (5.55-5.63)[c]	3.43 (3.33-3.56)[c]

[a] Three replicates were used for each test material for each organism.
[b] Log reduction in spores with range in parentheses.
[c] Surrogates significantly different from *B. antrhacis* for specified material (P≤0.05).

CDG Research Corporation – Chlorine Dioxide Statistical Analysis

Material		*B. anthracis*	*B. subtilis*	*G. stearothermophilus*
Porous	**Industrial-grade Carpet**	4.62	4.44	3.22
	Painted Concrete	7.25	4.74	5.79
	Bare Wood	4.33	4.48	3.79
Non-porous	**Glass**	5.70	5.23	3.87
	Decorative Laminate	4.57	5.14	4.44
	Painted Wallboard Paper	≥7.68	4.62	5.62
	Galvanized Metal Ductwork	≥7.79	5.57	3.43

All values are significantly different than zero ($P \leq 0.05$) except 

CDG Research Corporation – Chlorine Dioxide Statistical Analysis

Material		*B. anthracis*	*B. subtilis*	*G. stearothermophilus*
Porous	**Industrial-grade Carpet**	4.62	4.44	3.22
	Painted Concrete	7.25	4.74	5.79
	Bare Wood	4.33	4.48	3.79
Non-porous	**Glass**	5.70	5.23	3.87
	Decorative Laminate	4.57	5.14	4.44
	Painted Wallboard Paper	≥7.68	4.62	5.62
	Galvanized Metal Ductwork	≥7.79	5.57	3.43

Mean value is significantly different than *B. anthracis* (P≤0.05)

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